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Structural and Synthetic Studies on Maleic Anhydride and Related Diacid Natural Products.

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Email address: chris.willis@bristol.ac.uk We dedicate this paper to Professor Steve Davies in recognition of his extensive contributions to science and the wider community

Abstract: A concise approach has been developed for the total syntheses of tricladic acids A, B and C previously isolated from *Tricladium castaneicola* confirming the structures of these fungal natural products. The required (*E*)-1,3-diene scaffolds were generated with complete stereocontrol *via* coupling of the appropriate nitronate with an unsaturated bromo-diester which was prepared in 2 steps from citraconic anhydride. The reported structures of the anhydride waquafrane B and the parent diacid were synthesised leading to revision of the originally proposed structure of the natural product and, in accord with Sutherland, confirming that anhydrides with *exo*-dienes are unstable. Biomimetic dimerisations of the proposed monomers of the nonadride scytalidin were investigated.

1. Introduction

Maleidride natural products, characterised by a medium-ring carbocycle with at least one fused maleic anhydride moiety, are a large family of polyketide fungal metabolites which possess a range of herbicidal and fungicidal properties. Byssochlamic acid **1** from *Byssochlamys fulva*,¹⁻⁶ and glauconic acid **2** and glaucanic acid **3** both from *Penicillium glaucum* were amongst the first members of the family to be isolated.^{7,8} Since then many more nonadrides have been reported including cornexistin **4** from *Paecilomyces variotii* which has generated significant interest as a potential wide-spectrum herbicide, due to its broad range of bioactivity and high tolerance by *Zea mays*.^{9,10} Scytalidin **5** isolated from *Scytalidium album* has been shown to be active against a wide-range of wood rotting pathogens, whilst higher plants are unharmed.¹¹⁻¹³ In addition, the rubratoxins *e.g.* rubratoxin B **7** have been investigated for their anti-tumour properties.¹⁴ Further examples of nonadrides include castaneolide **6** and heveadride **8**.^{15,16}

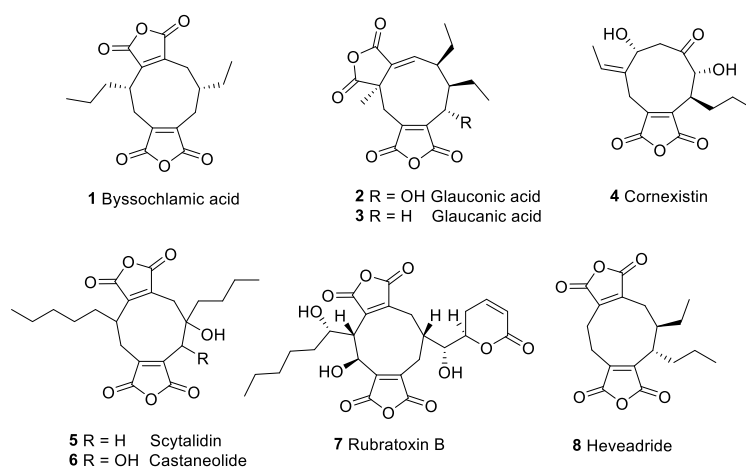
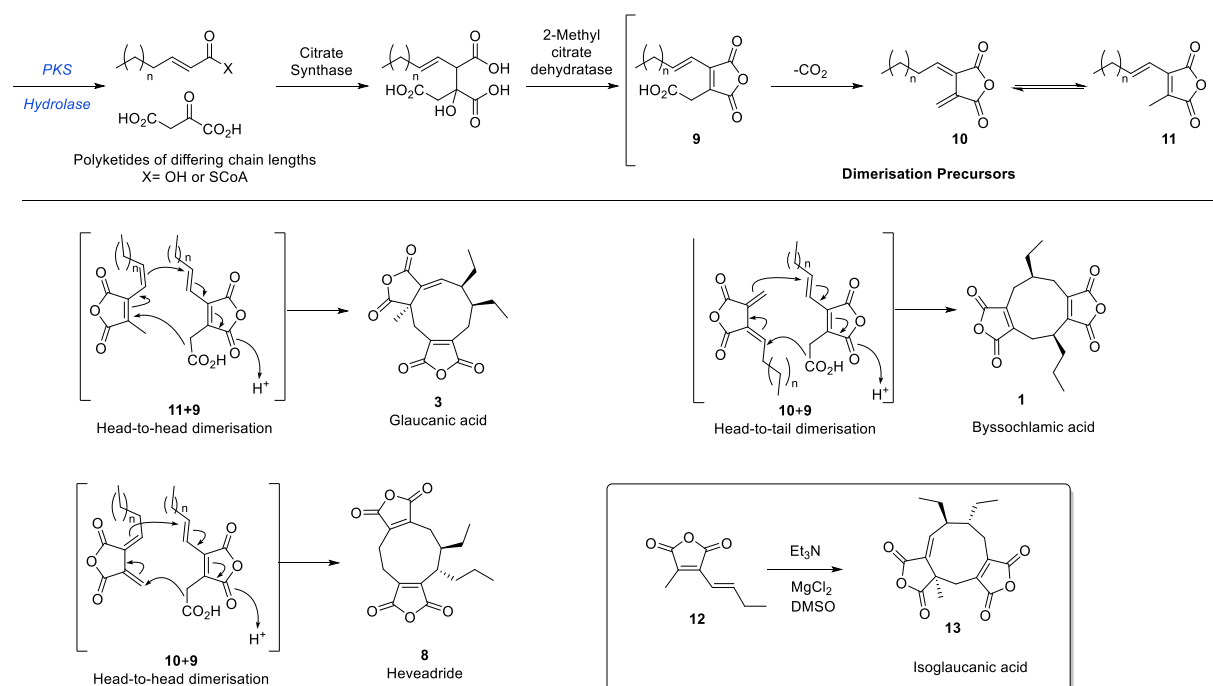


Figure 1. Examples of nonadrides

In 1965 Barton and Sutherland¹⁷ proposed that the biosynthesis of **1**, **2** and **3** proceeds *via* dimerisation of a C₉- anhydride monomer with different substitution patterns on the core framework arising from either “head-to-tail” or “head-to head” couplings and was supported by the results of isotopic labelling studies.^{18,19} More recent work has focussed on genome sequencing, knock out experiments and heterologous expression combined with isolation of intermediates leading to further insights into the biosynthesis of nonadrides.^{20–25} Using heterologous expression in *Aspergillus oryzae* we have shown that the precursors for cyclisation are formed by an iterative highly reducing polyketide synthase supported by a hydrolase together with citrate-processing enzymes (Scheme 1). Carboxylic acids such as **9** have been shown to undergo spontaneous decarboxylation, yielding **11**, and both isomers **10** and **11** may be formed in this fashion.²⁶ Various dimerisation modes of **9** (or the decarboxylated monomer **11**) with either itself or with **10** may then give rise to the carbocyclic ring of the nonadrides, with different substitution patterns obtained.



Scheme 1 Proposed biosynthesis of nonadrides and a biomimetic dimerisation

Recreation of this dimerisation step *in vitro* is an attractive strategy for the total synthesis of nonadrides. Early work by Sutherland showed that treatment of **12** with Et₃N in DMF could promote cyclisation giving *iso*-glaucanic acid **13** in 4% isolated yield,²⁷ and this work was further developed by Baldwin using Et₃N and MgCl₂ in DMSO which gave **13** along with further products.²⁸ Sulikowski tethered the two methylmaleic anhydride moieties together to enhance the efficiency of ring formation.^{29,30} In all cases, synthetic studies have focused on the homodimerisation of analogues of **11**. No studies have been reported on the proposed dimerisation pathways involving monomer **10** with the *exo*-diene leading to scaffolds found in heveadride **8** or byssochlamic acid **1**. This may be attributed, in part, to an early report from Sutherland which indicated that such compounds are unstable.²⁷

Pursuing an interest in the biosynthesis of maleidride natural products with fungicidal activities, herein we describe recent studies on the total syntheses of fungal dienoic acids tricladic acids A, B and C as well as the reported structure of waquafranone B isolated from *Wicklowia aquatica* and analogues of the *exo*-diene monomer **10** required for dimerisation studies.^{31,32}

2. Results and Discussion

2.1 Total synthesis of tricladic acids A, B and C

In 2015, a series of natural products including maleic anhydride derivatives tricladolides A-D **14-17** were isolated from the aquatic fungus *Tricladium castaneicola*.³¹ In addition, after an extensive purification protocol, three minor metabolites, tricladic acids A, B and C **18-20** were isolated and their structures tentatively assigned by spectroscopic methods and correlation with known compounds. The tricladic acids showed inhibitory activity against fungi such as *Phytophthora sp.* However, further investigations of their bioactivities were limited by both the difficulty in culturing aquatic hyphomycetes such as *T. castaneicola*,^{33,34} and the low titres of isolated natural products.

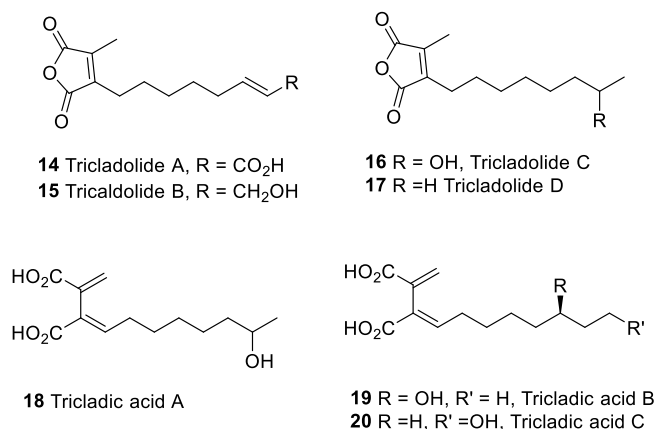
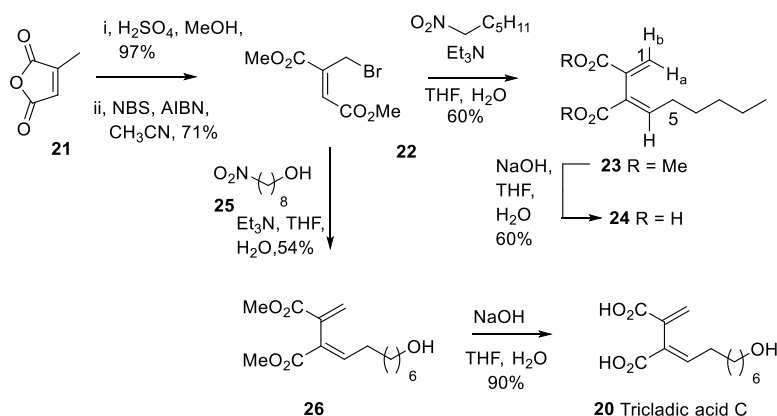


Figure 2. Natural products isolated from *T. castaneicola*

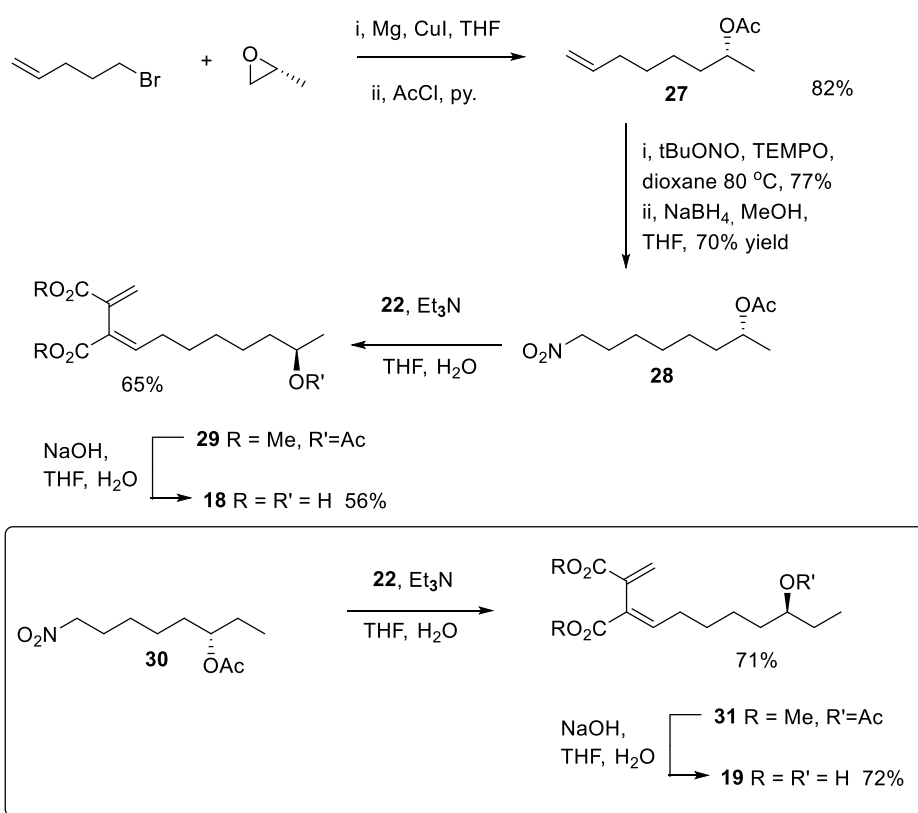
We devised a synthetic strategy to each of the target tricladic acids based on the nitronate additions to bromide **22** first reported by Amri for the preparation of similar 1,3-dienes.³⁵ Bromide **22** was prepared in 2 steps and 69% yield from citraconic anhydride **21** by esterification followed by Wohl-Ziegler bromination (Scheme 2). Reaction conditions were then optimised for the key coupling of bromide **22** and nitrohexane using different bases and solvents. The required diene **23** was isolated in 60% yield as a single isomer using Et₃N (4 equivalents) and slow addition of bromide **22** in THF:water (see ESI for full details). The *E*-geometry of the *endo* double bond of **23** was confirmed by NOESY studies (correlation between 5-H₂ and 1-H₃) and an EXSIDE experiment³⁶ which showed ³J_(CH) 7.5 Hz in accord with a *cis* relationship between the ester carbonyl and 4-H. Diester **23** was readily hydrolysed to the corresponding diacid **24** in 60% yield.



Scheme 2. Synthesis of monomer **24** and tricladic acid C **20**

This methodology was applied to the synthesis of each of the tricladic acids. Monobromination of octane 1,8-diol with HBr in toluene followed by displacement of the bromide with sodium nitrite gave the required alcohol **25** for the synthesis of tricladic acid C **20** (Scheme 2). Coupling **25** with bromide **22** followed by hydrolysis of the resultant diester **26** gave diacid **20** with spectroscopic data in accord with that reported for the natural product.³¹

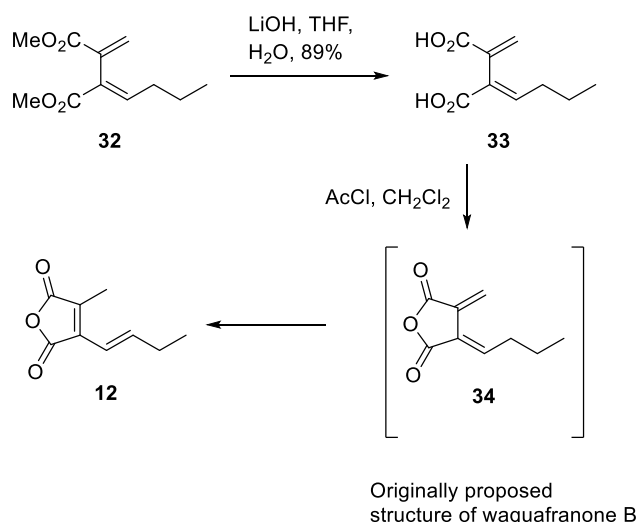
Tricladolide C **16** isolated from *T. castaneicola* was racemic as determined by the Mosher's method. By analogy, tricladic acid A **18** ($[\alpha]_D -0.4$, c 0.35, MeOH) was presumed to be close to racemic but not confirmed whilst tricladic acid B **19** was isolated as a 3:1 mixture of 9*S*:9*R* enantiomers, again determined by the Mosher's method. We developed approaches for their total syntheses which could be readily adapted for the preparation of either enantiomer of the natural products as shown in Scheme 3. A copper-catalysed addition of pent-4-enylmagnesium bromide to (*S*)-propylene oxide followed by acetylation of the resultant secondary alcohol gave unsaturated acetate **27**. Protection of the alcohol was required for the next stage, to prevent oxidation to the corresponding ketone as reported by Hu and co-workers.³⁷ The required (*R*)-nitro-acetate **28** was prepared in 54% yield using *t*-butyl nitrite and TEMPO followed by reduction with NaBH₄. A similar approach was used for the synthesis of (*S*)-nitro-acetate **30** starting from (*S*)-1,2-epoxybutane and 3-butenyl magnesium bromide giving **30** in 44% yield over the 4 steps. Reaction of bromide **22** separately with nitro-acetates **28** and **30** under the optimised conditions gave, after hydrolysis of their resultant dimethyl esters, (*R*)-tricladic acid A **18**, $[\alpha]_D -5$, c 0.91 MeOH, and (*S*)-tricladic acid B **19**. The spectroscopic data of both were in accord with those reported for the natural products isolated from *T. castaneicola*. The specific rotation for synthetic (*S*)-tricladic acid B **19**, $[\alpha]_D +4$, c 1.70, MeOH, was consistent with the proposal that the natural product was a 3:1 mixture of enantiomers in favour of the *S* enantiomer, $[\alpha]_D +2$, c 0.34, MeOH.



Scheme 3. Enantioselective synthesis of tricladic acids A and B **18** and **19**

2.2 Synthesis and structural revision of waquafranone B

In 2010 analogues of heveadride **8** were isolated from the fungus *Wicklowia aquatica* along with the natural product waquafranone B **34** (Scheme 4).³² The structure was assigned by spectroscopic methods as an anhydride **34** with an *exo*-diene. However, whilst the reported HREIMS gave $M^+168.0786$ in accord with the proposed molecular formula $C_9H_{12}O_3$, the IR spectrum showed a carbonyl stretch at 1685 cm^{-1} rather than as expected for an anhydride (*cf.* carbonyl stretches of the anhydrides tricladolides A-D **14-17**, ν_{max} *ca.* 1820 and 1770 cm^{-1}) but more in accord with tricladic acids A, B and C **18-20** (ν_{max} C=O, *ca.* 1695 cm^{-1}). Thus, both diacid **33** and anhydride **34** were synthesised. Coupling 1-nitrobutane with bromide **22** followed by hydrolysis of the resultant diester **32** gave the required diacid **33**. Treatment of **33** with $\text{AcCl}/\text{CH}_2\text{Cl}_2$ at $-30\text{ }^\circ\text{C}$ gave anhydride **34** which was unstable even when kept at $-78\text{ }^\circ\text{C}$ and after 96 h was converting to a mixture of products including maleic anhydride **12**. This was in accord with Sutherland who had reported that such anhydrides are unstable.²⁷ The ^1H -NMR spectrum (CDCl_3) of anhydride **34** displayed alkenic signals at δ 7.24, 6.59 and 6.13 whereas for waquafranone B these were reported to resonate at δ 6.97, 6.45 and 5.62. The ^1H -NMR spectrum of diacid **33** was found to be pH dependent and in CDCl_3 + 1 equivalent of DABCO gave alkenic signals at δ 6.96, 6.43 and 5.56 which correlated well with the natural product. Furthermore, the ^{13}C -NMR, IR and MS data were fully consistent with the reported data and hence we conclude that waquafranone B is diacid **33**.

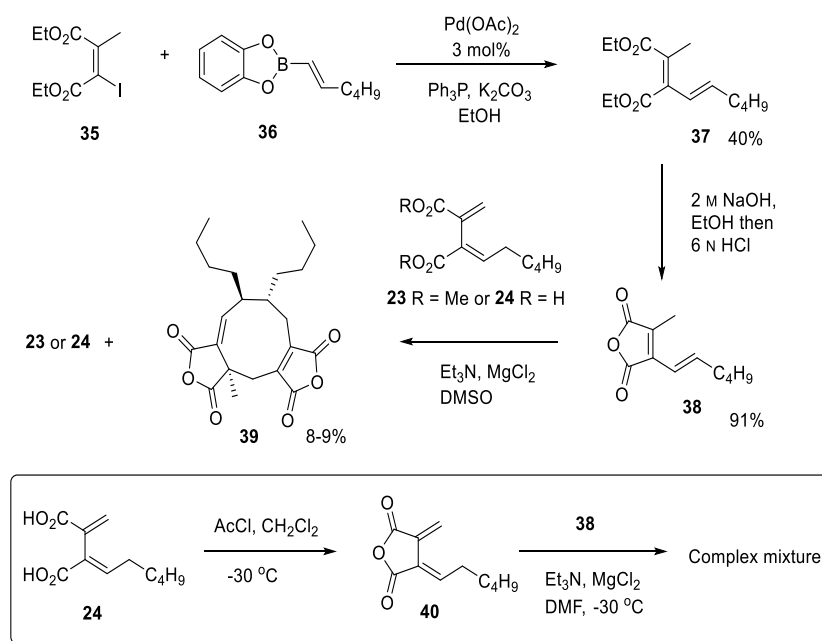


Scheme 4. Synthesis of diacid **33** and unstable anhydride **34**

2.3 Synthesis of proposed monomers for scytalidin biosynthesis and biomimetic dimerisations

As anhydride **34** was unstable there was a concern that analogues may not be good substrates for the proposed “head-to-tail” dimerisations to byssoclamic acid **1** or scytalidin **5** scaffolds (Scheme 1) but the corresponding diacids or dimethyl esters may be alternatives. To examine the proposed “head-to-tail dimerisation”, scytalidin monomer **38** was prepared by modification of the approach of Baldwin and co-workers (Scheme 5).³⁸ Thus, a solvent-free hydroboration of hex-1-yne with catecholborane in the presence of dimethylacetamide gave catecholboronic ester **36** which was used without purification in a Suzuki coupling with the known³⁸ iodide **35** to give diene **37**. Hydrolysis of **37** and ring closure under acidic conditions gave the required maleic anhydride derivative **38**. In a control reaction **38** was treated with Et_3N and MgCl_2 in DMSO for 16 hours at room temperature and *iso*-glaucanic acid analogue **39** was isolated in 10% yield in accord with the results of Baldwin.²⁸ When the reaction

was repeated using a 1:1 mixture of anhydride **38** and either dimethyl ester **23** or diacid **24**, homodimerisation product **39** was isolated in similar yields as in the control experiment and the other component, either **23** or **24**, was recovered unchanged. A similar result was achieved even when the concentration of **38** was kept low by slow addition via a syringe pump.



Scheme 5. Synthesis of maleic anhydride **38** and biomimetic dimerisations

Next anhydride **40** with the *exo*-diene was prepared by treatment of diacid **24** with AcCl in CH_2Cl_2 at -30°C and used immediately in the dimerisation reaction with maleic anhydride **38** at -30°C giving a very complex mixture of products. Neither starting material (**38** and **40**) nor any clean products could be isolated.

3. Conclusion

In conclusion, a concise approach has been developed for the total syntheses of the tricladic acids A, B and C **16**, **17** and **18** isolated from *T. castaneicola* confirming the structures of the natural products. Coupling the appropriate nitronate with bromide **22** generated the required (*E*)-1,3-diene scaffolds with complete stereocontrol. This approach was used for the synthesis of the reported structure, anhydride **34**, of waquafranone B as well as the parent diacid **33** which led to reassignment of the structure of the natural product. In accord with Sutherland, we found that anhydrides with an *exo*-1,3-diene were unstable. Biomimetic “head-to-tail” dimerisations of monomer **38** with either diester **23** or diacid **24** lead to homodimerisation of **38** giving isoglaucanic acid analogue **39** and recovered **23** or **24** whereas reaction of **38** and **40** led to a complex mixture of products. Such dimerisations would need significant optimisation if to be of value in the synthesis of nonadrides.

4. Experimental Section

4.1 General Experimental

All reagents were sourced from commercial suppliers and were used without further purification unless stated otherwise. All reactions using anhydrous solvents were performed using standard Schlenk syringe-septa techniques, with flame dried glassware under a positive pressure of nitrogen. Anhydrous THF, Et₂O, hexane, toluene, acetonitrile and CH₂Cl₂ were dried by passing through a modified Grubbs system of alumina columns, manufactured by Anhydrous Engineering and stored over 3 Å molecular sieves. Dry DMF was obtained from Sigma-Aldrich, dry DMSO and acetone from Acros Organics, each were used without further purification or drying. Diisopropylamine and triethylamine were distilled over CaH₂ prior to use in moisture-sensitive reactions. All stated temperatures below ambient are the temperatures of the cooling baths, unless otherwise stated. Flash column chromatography was performed according to the procedures used by Still *et. al.*³⁹ using silica gel 60 (Fisher Scientific or Aldrich) and a suitable eluent. TLC analysis was performed with aluminium backed silica TLC plates (Merck-Kieselgel 60 F₂₅₄) with a suitable solvent system and was visualised using UV fluorescence (254 & 366 nm) and/or developed with potassium permanganate solution. NMR spectra of **18** and **19** were obtained after purification by HPLC (Phenomenex Kinetex C18 column, 250 × 21.2 mm, 5 µm pore size), eluting with 20-60% acetonitrile in water with 0.05% formic acid at 16 mL min⁻¹.

Infra-red spectra were recorded on a Perkin Elmer Spectrum 100 FTIR with an ATR accessory and frequencies are reported in wavenumbers (cm⁻¹). ¹H and ¹³C NMR spectra were recorded using Jeol ECS 400 MHz, Varian 400-MR (400 MHz), Varian VNMR500a (500 MHz), Varian VNMR500b (500 MHz), Bruker Advance III HD 500 Cryo (500 MHz), Varian VNMR S600 Cryo (600 MHz), and Bruker Avance III HD 700 (1.7mm micro-cryo) (700 MHz) spectrometers at ambient temperature. Chemical shifts (δ) are quoted in parts per million (ppm) and coupling constants (*J*) are in Hertz (Hz), rounded to 0.5 Hz intervals. Two-dimensional NMR techniques (HSQC, COSY, HMBC) were used routinely for structural assignment. Use of NOESY, TOCSY and EXSIDE techniques is indicated as appropriate. Residual solvent peaks were used as the internal reference for proton and carbon chemical shifts. HRMS ESI were performed on either a Bruker Daltonics Apex 4, 7 Tesla FTICR or microTOF II. Samples were submitted in MeOH or CH₂Cl₂. Specific rotations ($[\alpha]_D^{25}$) were measured on a Bellingham and Stanley Ltd. ADP220 polarimeter and are quoted in (° ml)(g dm)⁻¹.

Dimethyl (*E*)-2-hexylidene-3-methylenesuccinate **23**

1-Nitrohexane (180 mg, 1.37 mmol) and triethylamine (440 mg, 4.3 mmol) were dissolved in a mixture of H₂O (7 ml) and THF (7 ml). The solution was stirred for 2 h at room temperature. Allyl bromide **22** (249 mg, 1.05 mmol) dissolved in THF (7 ml) was added over 2 hours at 0 °C. The resultant orange solution was stirred for 16 h at room temperature then diluted with water (10 ml). The aqueous layer was extracted with diethyl ether (3 × 75 ml). The organic extracts were combined and concentrated *in vacuo*. The residue was purified by column chromatography using ethyl acetate/petroleum ether (1-4% ethyl acetate) to give **23** as a colourless oil (152 mg, 60%). δ_H (400 MHz, CDCl₃) 6.99 (1H, *t*, *J* 7.0, 4-H), 6.49 (1H, *d*, *J* 1.5, 1-*HH*), 5.58 (1H, *d*, *J* 1.5, 1-*HH*), 3.74 (3H, *s*, OCH₃), 3.72 (3H, *s*, OCH₃), 2.16 (2H, *app. q*, *J* 7.5, 5-H₂), 1.48- 1.38 (2H, *m*, 6-H₂), 1.33-1.23 (4H, *m*, 7-H₂ and 8-H₂), 0.87 (3H, *m*, 9-H₃); δ_C (100 MHz, CDCl₃) 166.9 (3-C=O), 166.6 (2-C=O), 146.7 (C-4), 135.4, 129.9 (C-2 and C-3), 129.5 (C-1), 52.3 (OCH₃), 52.1 (OCH₃), 31.6 (C-7), 29.6 (C-5), 28.5 (C-6), 22.5 (C-8), 14.1 (9-H₃); ν_{max} (film) 2953, 2928, 1719, 1435, 1246, 1199, 1065; HRMS (ESI) calc for C₁₃H₂₁O₄ [M+H] 241.1434, found 241.1436.

(E)-2-Hexylidene-3-methylenesuccinic acid **24**

Diester **23** (295 mg, 1.20 mmol) was dissolved in ethanol (12 mL) and sodium hydroxide (18 mL, 2 M) added. The reaction mixture was stirred at room temperature for 16 h, then aqueous sodium sulfate (4%, 25 mL) added and the reaction mixture acidified to pH 1 with hydrochloric acid (6 M). The aqueous mixture was extracted with diethyl ether (4 × 20 mL), and the combined organic layers dried over MgSO₄, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol/acetic acid (25-40% ethyl acetate, 1% acetic acid) to yield diacid **24** as a white solid (158 mg, 60%). δ_{H} (400 MHz, MeOD) 6.95 (1H, t, *J* 7.5, 4-H), 6.44 (1H, s, 1-HH), 5.60 (1H, s, 1-HH), 2.18 (2H, app. q, *J* 7.5, 5-H₂), 1.46 (2H, m, 6-H₂), 1.38-1.25 (4H, m, 7-H₂ and 8-H₂), 0.90 (3H, t, *J* 6.5, 9-H₃); δ_{C} (100 MHz, MeOD) 168.2 (C=O), 167.9 (C=O), 145.6 (C-4), 136.4 (C-3), 130.8 (C-2), 128.3 (C-1), 31.2 (C-7), 29.0 (C-5), 28.0 (C-6), 22.0 (C-8), 12.9 (C-9); ν_{max} (film) 2956, 2928, 2859, 2650, 1683, 1620, 1426, 1281; HRMS (ESI) calc for C₁₁H₁₆NaO₄ [M+Na]⁺ 235.0941, found 235.0930.

Dimethyl (E)-2-(8-hydroxyoctylidene)-3-methylenesuccinate **26**

Nitroalkanol **25** (251 mg, 1.43 mmol, 1.3 equiv.) and triethylamine (0.54 mL, 0.39 g, 3.8 mmol, 3.5 equiv.) were dissolved in THF (7.5 mL) and water (7.5 mL) and stirred for 2 h. Bromide **22** (263 mg, 1.10 mmol, 1 equiv.) in THF (3 mL) was added at 0 °C, and the reaction mixture stirred for an additional 16 h at room temperature. The orange solution was diluted with water (20 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (25-50% ethyl acetate) to yield diester **26** (167 mg, 54%) as a colourless oil. δ_{H} (400 MHz, CDCl₃) 6.91 (1H, t, *J* 7.5, 4-H), 6.41 (1H, d, *J* 1.5, 1-HH), 5.51 (1H, d, *J* 1.5, 1-HH), 3.66 (3H, s, OCH₃), 3.64 (3H, s, OCH₃), 3.51 (2H, t, *J* 6.5, 11-H₂), 2.26 (1H, s, OH), 2.09 (2H, q, *J* 7.5, 5-H₂), 1.46 (2H, m, 10-H₂), 1.36 (2H, p, *J* 7.0, 6-H₂), 1.29-1.19 (6H, m, 7-H₂, 8-H₂, 9-H₂); δ_{C} (100 MHz, CDCl₃) 166.7 (3-C=O), 166.4 (2-C=O), 146.5 (C-4), 135.1 (C-2), 129.7 (C-3), 129.5 (C-1), 62.6 (C-11), 52.1 (OCH₃), 51.9 (OCH₃), 32.6 (C-10), 29.4 (C-5), 29.2, 29.1, 28.5, 22.6 (C-6, C-7, C-8 and C-9); ν_{max} (film) 3515, 2931, 2857, 1714, 1437, 1255, 908, 730; HRMS (ESI) calc for C₁₅H₂₄NaO₅ [M+Na]⁺ 307.1516, found 307.1514.

(E)-2-(8-Hydroxyoctylidene)-3-methylenesuccinic acid (Tricladic Acid C) **20**

Diester **26** (150 mg, 0.53 mmol) was dissolved in ethanol (15 mL) and sodium hydroxide (15 mL, 2 M) added. The reaction mixture was stirred at room temperature for 16 h, then aqueous sodium sulfate (4%, 40 mL) added and the reaction mixture acidified to pH 1 with hydrochloric acid (6 M). The aqueous mixture was extracted with diethyl ether (4 × 25 mL), and the combined organic layers dried over MgSO₄, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol/acetic acid (75% ethyl acetate, 1% acetic acid) to give diacid **20** (120 mg, 90%) as a colourless oil. δ_{H} (400 MHz, MeOD) 6.92 (1H, t, *J* 7.5, 4-H), 6.42 (1H, d, *J* 1.5, 1-HH), 5.57 (1H, d, *J* 1.5, 1-HH), 3.50 (2H, t, *J* 6.5, 11-H₂), 2.16 (2H, q, *J* 7.5, 5-H₂), 1.52-1.39 (4H, m, 6-H₂ and 10-H₂), 1.38-1.22 (6H, m, 7-H₂, 8-H₂ and 9-H₂); δ_{C} (100 MHz, MeOD) 169.6 (3-C=O), 169.3 (2-C=O), 147.1 (C-4), 137.7 (C-2), 132.1 (C-3), 129.8 (C-1), 62.9 (C-11), 33.5 (C-10), 30.4 (C-5), 30.3, 30.2 (C-7 and C-8), 29.6 (C-6), 26.7 (C-9); ν_{max} (film) 2931, 2858, 2489, 2230, 2072, 1686, 1621, 1329, 1117, 1056, 971; HRMS (ESI) calc for C₁₃H₁₉O₅ [M-H]⁻ 255.1238, found 255.1235. NMR data in accordance with literature of the isolated natural product.³¹

(R)-2-Acetoxyoct-7-ene **27**

5-Bromo-1-pentene (4.6 mL, 5.80 g, 39 mmol) was dissolved in dry THF (30 mL). A small portion of which was added to a suspension of magnesium turnings (1.80 g, 75 mmol) in THF (45 mL) under a nitrogen atmosphere. The solution was sonicated for 5 minutes to initiate the Grignard reaction. Once

initiated, the remaining 5-bromo-1-pentene was added dropwise over 20 minutes. The reaction mixture was stirred for 1 h. The reaction mixture was cooled to $-30\text{ }^{\circ}\text{C}$ before CuI (150 mg, 0.78 mmol) was added. (*R*)-Propylene oxide (1.05 mL, 0.87 g, 15.0 mmol) was added and the reaction was warmed to $-15\text{ }^{\circ}\text{C}$. The reaction was stirred for a further 2 h and then quenched with saturated aqueous NH_4Cl (10 mL). After warming to room temperature, diethyl ether was added (30 mL) and the layers were separated. The organic layer was washed sequentially with water (30 mL) and brine (30 mL). The combined aqueous layers were extracted with diethyl ether ($3 \times 75\text{ mL}$). The organic extracts were combined, dried over MgSO_4 and concentrated *in vacuo*. The crude residue and acetyl chloride (2.10 mL, 2.30 g, 30.0 mmol) were dissolved in diethyl ether (45 mL). Pyridine (2.4 mL, 2.4 g, 30 mmol) was added to the reaction mixture resulting in the formation of a white suspension. The solution was heated to reflux at $35\text{ }^{\circ}\text{C}$ until TLC analysis (10% ethyl acetate, hexane) indicated that the starting material was consumed (1 h). After cooling the reaction mixture to room temperature, aqueous HCl (1 M, 40 mL) was added. The aqueous layer was separated and extracted with diethyl ether ($3 \times 75\text{ mL}$). The organic extracts were combined, dried over MgSO_4 , filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography using petroleum ether/ethyl acetate (3% ethyl acetate) to give **27** as a colourless oil (2.10 g, 82%); $[\alpha]_{\text{D}}^{22} = +3.0$ (c 1.00, CHCl_3); δ_{H} (400 MHz, CDCl_3) 5.73 (1H, ddt, *J* 17.0, 10.0, 6.5, 7-H), 4.93 (1H, dq, *J* 17.0, 2.0, 8-HH), 4.87 (1H, ddt, *J* 10.0, 2.0, 1.5, 8-HH), 4.62 (1H, sext, *J* 6.0, 2-H), 1.98 (2H, m, 6-H₂), 1.96 (3H, s, 10-H₃), 1.54-1.21 (6H, m, 3-H₂, 4-H₂, 5-H₂), 1.13 (3H, d, *J* 6.0, 1-H₃); δ_{C} (100 MHz, CDCl_3) 170.8 (C-9), 138.7 (C-7), 114.4 (C-8), 71.0 (C-2), 35.7 (C-3), 33.6 (C-6), 28.7 (C-5), 24.8 (C-4), 21.4 (C-10), 20.0 (C-1); ν_{max} (film) 3074, 2977, 2932, 1735, 1640, 1240; HRMS (ESI) calc for $\text{C}_{10}\text{H}_{18}\text{O}_2\text{Na}$ $[\text{M}+\text{Na}]^+$ 193.1199, found 193.1194.

(*R,E*)-2-Acetoxy-8-nitrooct-7-ene

Acetylated alcohol **27** (1.20 g, 7.00 mmol), *tert*-butyl nitrite (1.80 mL, 1.60 g, 15.0 mmol) and TEMPO (268 mg, 1.72 mmol) were dissolved in 1,4-dioxane (18 mL) and heated to reflux in air at $90\text{ }^{\circ}\text{C}$ for 16 h. The orange solution was washed through a Celite® pad, concentrated *in vacuo* and purified by column chromatography using petroleum ether/diethyl ether (14-25% diethyl ether) to give the title compound as an orange oil (1.16 g, 77%). $[\alpha]_{\text{D}}^{22} = +14$ (c 1.00, CHCl_3); δ_{H} (400 MHz, CD_2Cl_2) 7.18 (1H, dt, *J* 13.0, 7.0, 7-H), 6.91 (1H, dt, *J* 13.0, 1.5, 8-H), 4.78 (1H, sext, *J* 6.5, 2-H), 2.19 (2H, qd, *J* 7.0, 1.5, 6-H₂), 1.91 (3H, s, 10-H₃), 1.55-1.25 (6H, m, 3-H₂, 4-H₂, 5-H₂), 1.10 (3H, d, *J* 6.0, 1-H₃); δ_{C} (100 MHz, CD_2Cl_2) 170.4 (C-9), 142.6 (C-7), 139.6 (C-8), 70.4 (C-2), 35.4 (C-3), 28.3 (C-6), 27.5 (C-5), 24.8 (C-4), 21.1 (C-10), 19.7 (C-1); ν_{max} (film) 2977, 2936, 1729, 1648, 1522, 1351, 1242; HRMS (ESI) calc for $\text{C}_{10}\text{H}_{17}\text{NO}_4\text{Na}$ $[\text{M}+\text{Na}]^+$ 238.1055, found 238.1056.

(*R*)-2-Acetoxy-8-nitrooctane **28**

To a solution of the above nitroalkene (400 mg, 1.86 mmol) in THF:methanol (10:1, 18 mL) at $0\text{ }^{\circ}\text{C}$ under a nitrogen atmosphere, NaBH_4 (140 mg, 3.72 mmol) was added in five portions. The reaction was stirred for 1 h at $0\text{ }^{\circ}\text{C}$. the resultant orange solution was quenched with saturated aqueous NaHCO_3 (5 mL), diluted with water (50 mL) and extracted with ethyl acetate ($4 \times 50\text{ mL}$). The combined organic layers were washed with brine (10 mL), dried over MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by column chromatography using hexane/ethyl acetate (12% ethyl acetate) to give nitroalkane **28** as a colourless oil (284 mg, 70%). $[\alpha]_{\text{D}}^{22} = +21$ (c 0.66, CHCl_3); δ_{H} (400 MHz, CDCl_3) 4.88 (1H, sext, *J* 6.5, 2-H), 4.37 (2H, t, *J* 7.5, 8-H₂), 2.02 (3H, s, 10-H₃), 2.00 (2H, quin, *J* 7.5, 7-H₂), 1.60-1.27 (8H, m, 3-H₂, 4-H₂, 5-H₂, 6-H₂), 1.20 (3H, d, *J* 6.5, 1-H₃); δ_{C} (100 MHz, CDCl_3) 170.8 (C-9), 75.6 (C-8), 70.8 (C-2), 35.7 (C-3), 28.6 (C-5), 27.3 (C-7), 26.1 (C-6), 25.0 (C-4), 21.4 (C-10), 20.0 (C-1); ν_{max} (film) 2975, 2860, 2933, 1731, 1552, 1372, 1244; HRMS (ESI) calc for $\text{C}_{10}\text{H}_{19}\text{NO}_4\text{Na}$ $[\text{M}+\text{Na}]^+$ 240.1212, found 240.1209.

Dimethyl (*R,E*)-2-(6-acetoxyheptylidene)-3-methylenesuccinate **29**

Nitroalkane **28** (200 mg, 0.92 mmol) and triethylamine (0.58 mL, 4.10 mmol) were dissolved in THF (8 mL) and water (8 mL). The solution was stirred for 2 h at room temperature. Allyl bromide **22** (175 mg, 0.74 mmol) in THF (2 mL) was added over 2 h at 0 °C with a syringe pump and stirred for an additional 16 h at room temperature. The yellow solution was diluted with water (30 mL) and extracted with diethyl ether (4 × 75 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography using petroleum ether/ethyl acetate (9-17% ethyl acetate) to give triester **29** as a colourless oil (150 mg, 65%). $[\alpha]_D^{22} = +16$ (c 0.66, CHCl₃); δ_H (400 MHz, CDCl₃) 6.97 (1H, t, *J* 7.5, 4-H), 6.49 (1H, d, *J* 1.5, 1-HH), 5.57 (1H, d, *J* 1.5, 1-HH), 4.85 (1H, sext, *J* 6.5, 10-H), 3.74 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 2.16 (2H, q, *J* 7.5, 5-H₂), 2.01 (3H, s, 13-H₃), 1.59-1.38 (4H, m, 7-H₂, 9-H₂), 1.34-1.25 (4H, m, 6-H₂, 8-H₂), 1.18 (3H, d, *J* 6.5, 11-H₃); δ_C (100 MHz, CDCl₃) 170.7 (C-12), 166.7 (3-C=O), 166.4 (2-C=O), 146.2 (C-4), 135.2 (C-2), 129.9 (C-3), 129.4 (C-1), 70.9 (C-10), 52.2 (2-OCH₃), 52.0 (3-OCH₃), 35.8 (C-9), 29.4 (C-5), 29.1 (C-6), 28.5 (C-7), 25.1 (C-8), 21.4 (C-13), 19.9 (C-11); ν_{max} (film) 2934, 2859, 1721, 1623, 1242, 1199; HRMS (ESI) calc for C₁₇H₂₆O₆Na [M+Na]⁺ 349.1627, found 349.1625.

(*R,E*)-2-(6-Hydroxyheptylidene)-3-methylenesuccinic acid (Tricladic acid A) **18**

Triester **29** (140 mg, 0.45 mmol) was dissolved in ethanol (5 mL) and added to aqueous sodium hydroxide (10 mL, 2 M) at 0 °C over 20 minutes. The solution was stirred at room temperature for 16 h. The reaction mixture was acidified to pH 1 with aqueous HCl (6 M). The solution was extracted with diethyl ether (4 × 50 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography using petroleum ether/ethyl acetate/acetic acid (70% ethyl acetate, 0.8% acetic acid) to give (**R**)-**18** as a viscous oil (115 mg, 56%). $[\alpha]_D^{22} = -5$ (c 0.91, MeOH) [lit. $[\alpha]_D^{20} -0.4$ (c 0.35, MeOH)]³¹; δ_H (400 MHz, CH₃OD) 6.96 (1H, t, *J* 7.5, 4-H), 6.43 (1H, d, *J* 1.5, 1-HH), 5.59 (1H, d, *J* 1.5, 1-HH), 3.70 (1H, m, 10-H), 2.21 (2H, q, *J* 7.5, 5-H₂), 1.50-1.25 (8H, m, 6-H₂, 7-H₂, 8-H₂, 9-H₂), 1.14 (3H, d, *J* 6.0, 11-H₃); δ_C (100 MHz, CH₃OD) 168.6 (3-C=O), 168.3 (2-C=O), 145.3 (C-4), 136.9 (C-2), 131.2 (C-3), 128.0 (C-1), 67.1 (C-10), 38.6 (C-9), 29.1 (C-5), 29.0 (C-7), 28.3 (C-6), 25.2 (C-8), 22.1 (C-11); ν_{max} (film) 3385, 2931, 2858, 1693, 1621, 1279, 1130; HRMS (ESI) calc for C₁₃H₂₀O₅Na [M+Na]⁺ 279.1208, found 279.1208. ¹H and ¹³C spectra obtained after further purification by preparative HPLC, and is in accordance with literature.³¹

(*S,E*)-3-Acetoxy-8-nitrooct-7-ene

(*S*)-3-Acetoxyoct-7-ene (1.35 g, 7.93 mmol), *tert*-butyl nitrite (2.0 mL, 1.2 g, 16.0 mmol) and TEMPO (513 mg, 3.28 mmol) were dissolved in 1,4-dioxane (20 mL) and heated to reflux in air at 90 °C for 16 h. The orange solution was washed through a Celite® pad, concentrated *in vacuo*, and purified by column chromatography using petroleum ether/diethyl ether (14-25% diethyl ether) to give the title compound as an orange oil (1.18 g, 69%). $[\alpha]_D^{22} = -11$ (c 1.00, CHCl₃); δ_H (400 MHz, CDCl₃) 7.17 (1H, dt, *J* 13.5, 7.5, 7-H), 9.91 (1H, dt, *J* 13.5, 1.5, 8-H), 2.20 (2H, m, 6-H₂), 1.94 (3H, s, 10-H₃), 1.53-1.39 (6H, m, 2-H₂, 4-H₂, 5-H₂), 0.80 (3H, t, *J* 7.5, 1-H₃); δ_C (100 MHz, CDCl₃) 170.6 (C-9), 142.3 (C-7), 139.8 (C-8), 74.5 (C-3), 33.0 (C-4), 28.2 (C-6), 27.0 (C-2), 23.6 (C-5), 20.7 (C-10), 9.3 (C-1); ν_{max} (film) 2939, 2878, 1728, 1649, 1522, 1351, 1241; HRMS (ESI) calc for C₁₀H₁₇NO₄Na [M+Na]⁺ 238.1055, found 238.1057.

(*S,E*)-3-Acetoxy-8-nitrooctane **30**

To a solution of the above nitroalkene (1.00 g, 4.60 mmol) in THF:methanol (10:1, 45 mL) at 0 °C under a nitrogen atmosphere, NaBH₄ (263 mg, 6.97 mmol) was added in five portions. The reaction was stirred for 1 h at 0 °C. the resultant red solution was quenched with saturate aqueous NaHCO₃ (10 mL),

diluted with water (100 mL) and extracted with ethyl acetate (4 × 75 mL). The combined organic layers were washed with brine (30 mL), dried MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography using hexane/ethyl acetate (10-12% ethyl acetate) to give nitroalkane **30** as a colourless oil (706 mg, 70%). $[\alpha]_D^{22} = -11$ (c 1.00, CHCl₃); δ_H (400 MHz, CDCl₃) 4.73 (1H, quin, *J* 6.0, 3-H), 4.31 (2H, t, *J* 7.0, 8-H₂), 1.98 (3H, s, 10-H₃), 1.94 (2H, quin, *J* 7.5, 7-H₂), 1.55-1.42 (4H, m, 2-H₂, 4-H₂), 1.38-1.22 (4H, m, 5-H₂, 6-H₂), 0.81 (3H, t, *J* 7.5, 1-H₃); δ_C (100 MHz, CDCl₃) 170.9 (C-9), 75.5 (C-8), 75.0 (C-3), 33.3 (C-4), 27.3 (C-7), 27.0 (C-2), 26.1 (C-6), 24.6 (C-5), 21.2 (C-10), 9.6 (C-1); ν_{max} (film) 2938, 2864, 1727, 1550, 1372, 1241; HRMS (ESI) calc for C₁₀H₁₉NO₄Na [M+Na]⁺ 240.1212, found 240.1208.

Dimethyl (*S, E*)-2-(5-acetoxyheptylidene)-3-methylenesuccinate **31**

Nitroalkane **30** (300 mg, 1.38 mmol, 1.1 equiv.) and triethylamine (0.87 mL, 6.20 mmol, 5 equiv.) were dissolved in THF (12 mL) and water (12 mL). Following 2 h of stirring a red solution was observed. Bromide **22** (285 mg, 1.20 mmol) in THF (3 mL) was added over 2 h at 0 °C with a syringe pump and stirred for an additional 14 h at room temperature. The yellow solution was diluted with water (40 mL) and extracted with diethyl ether (4 × 75 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using petroleum ether/ethyl acetate (9-17% ethyl acetate) to give triester **31** as a colourless oil (266 mg, 71%). $[\alpha]_D^{22} = -8$ (c 1.00, CHCl₃); δ_H (400 MHz, CDCl₃) 6.97 (1H, t, *J* 7.5, 4-H), 6.49 (1H, d, *J* 1.5, 1-HH), 5.56 (1H, d, *J* 1.5, 1-HH), 4.78 (1H, quin, *J* 6.0, 9-H), 3.74 (3H, s, OCH₃), 3.72 (3H, s, OCH₃), 1.56-1.40 (6H, m, 6-H₂, 8-H₂, 10-H₂), 1.36-1.23 (2H, m, 7-H₂), 0.86 (3H, t, *J* 7.5, 11-H₃); δ_C (100 MHz, CDCl₃) 170.9 (C-12), 166.7 (C=O), 166.4 (C=O), 146.0 (C-4), 135.2 (C-2), 130.0 (C-3), 129.5 (C-1), 75.2 (C-9), 52.2, 52.0 (2-OCH₃ and 3-OCH₃), 33.3 (C-8), 29.4 (C-5), 28.5 (C-6), 27.0 (C-10), 25.1 (C-7), 21.2 (C-13), 9.6 (C-11); ν_{max} (film) 2948, 2860, 1721, 1623, 1240; HRMS (ESI) calc for C₁₇H₂₆O₆Na [M+Na]⁺ 349.1627, found 349.1632.

(*S, E*)-2-(6-Hydroxyoctylidene)-3-methylenesuccinic acid ((*S*)-Tricladic acid B) **19**

Triester **31** (210 mg, 0.67 mmol) was dissolved in ethanol (7 mL) and added to aqueous sodium hydroxide (14 mL, 2 M) at 0 °C over 0.3 h. The solution was stirred at room temperature for 16 h. The reaction mixture was acidified to pH 1 with aqueous hydrochloric acid (6 M). The solution was extracted with ethyl acetate (4 × 75 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography using petroleum ether/ethyl acetate/acetic acid (70% ethyl acetate, 0.8% acetic acid) to give **19** as a viscous oil (124 mg, 72%). $[\alpha]_D^{22} = +4$ (c 1.70, MeOH); δ_H (400 MHz, CDCl₃) 6.96 (1H, t, *J* 7.5, 4-H), 6.44 (1H, d, *J* 1.5, 1-HH), 5.61 (1H, d, *J* 1.5, 1-HH), 3.43 (1H, m, 9-H), 2.21 (2H, q, *J* 7.5, 5-H₂), 1.50-1.25 (8H, m, 6-H₂, 7-H₂, 8-H₂, 10-H₂), 1.14 (3H, t, *J* 7.5, 11-H₃); δ_C (100 MHz, CDCl₃) 168.4 (3-C=O), 168.1 (2-C=O), 145.4 (C-4), 136.7 (C-2), 131.0 (C-3), 128.2 (C-1), 72.3 (C-9), 36.2 (C-8), 29.7 (C-10), 29.1 (C-5), 28.4 (C-6), 25.1 (C-7), 9.0 (C-11); ν_{max} (film) 3371, 2933, 2860, 1691, 1622, 1266, 1129; HRMS (ESI) calc for C₁₃H₂₀O₅Na [M+Na]⁺ 279.1208, found 279.1205. ¹H and ¹³C spectra obtained after further purification by preparative HPLC, and is in accordance with literature for the natural product.³¹

Dimethyl (*E*)-2-butyldiene-3-methylenesuccinate **32**

1-Nitrobutane (425 mg, 4.11 mmol, 1.3 equiv.) and triethylamine (2.20 mL, 1.60 g, 15.8 mmol, 5 equiv.) were dissolved in THF (20 mL) and water (20 mL) and stirred for 2 h. The reaction mixture was cooled to 0 °C, bromide **22** (750 mg, 3.21 mmol) in THF (7 mL) was added over 8 h, and then the reaction mixture stirred for an additional 16 h. The orange solution was diluted with water (20 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over MgSO₄, filtered,

and concentrated *in vacuo*. The crude material was purified by column chromatography using ethyl acetate/petrol (1-4% ethyl acetate) to give diester **32** as a colourless oil (314 mg, 47%). δ_{H} (400 MHz, CDCl_3) 6.97 (1H, t, J 7.5, 4-H), 6.46 (1H, d, J 1.5, 1-HH), 5.55 (1H, d, J 1.5, 1-HH), 3.71 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 2.12 (2H, q, J 7.5, 5- H_2), 1.44 (2H, q, J 7.5, 6- H_2), 0.89 (3H, t, J 7.5, 7- H_3); δ_{C} (100 MHz, CDCl_3) 166.8 (C=O), 166.5 (C=O), 146.4 (C-4), 135.3 (C-3), 130.0 (C-2), 129.5 (C-1), 52.2 (OCH_3), 52.0 (OCH_3), 31.6 (C-5), 22.0 (C-6), 13.9 (C-7); ν_{max} (film) 3416, 2959, 1935, 1719, 1459, 1145; HRMS (ESI) calc for $\text{C}_{11}\text{H}_{16}\text{NaO}_4$ $[\text{M}+\text{Na}]^+$ 235.0941, found 235.0945.

(*E*)-2-Butylidene-3-methylenesuccinic acid **33**

To a solution of diester **32** (500 mg, 2.4 mmol) in water (40 mL) and THF (40 mL) was added lithium hydroxide (1.13 g, 47 mmol, 20 equiv.). The reaction mixture was heated to 50 °C for 16 h, then cooled to room temperature. Saturated aqueous NaHCO_3 solution (15 mL) was added, then the reaction mixture was extracted with diethyl ether (3 \times 20 mL). The aqueous layer was acidified to pH 4 with solid citric acid, then extracted with diethyl ether (4 \times 20 mL). The combined organic layers were then extracted with water (5 \times 20 mL), dried over MgSO_4 , filtered, and concentrated *in vacuo* to give diacid **33** as an amorphous white solid (388 mg, 89%). δ_{H} (400 MHz, CDCl_3) 7.14 (1H, t, J 7.5, 4-H), 6.62 (1H, d, J 1.5, 1-HH), 5.71 (1H, d, J 1.5, 1-HH), 2.20 (2H, q, J 7.5, 5- H_2), 1.49 (2H, q, J 7.5, 6- H_2), 0.93 (3H, t, J 7.5, 7- H_3); δ_{C} (125 MHz, CDCl_3) 171.9 (C=O), 171.7 (C=O), 149.0 (C-4), 134.8 (C-3), 131.5 (C-3), 129.5 (C-1), 31.8 (C-5), 22.0 (C-6), 14.0 (C-7); ν_{max} (film) 2963, 2660, 2570, 1675, 1624, 1436, 1295, 1145; HRMS (ESI) calc for $\text{C}_9\text{H}_{12}\text{O}_4$ $[\text{M}-\text{H}]^-$ 183.0663, found 188.0659.

NMR on addition of 0.5 eq. of DABCO: δ_{H} (500 MHz, CDCl_3) 6.96 (1H, t, J 7.5, 4-H), 6.43 (1H, d, J 1.5, 1-HH), 5.56 (1H, d, J 1.5, 1-HH), 2.19 (2H, q, J 7.5, 5- H_2), 1.46 (2H, q, J 7.5, 6- H_2), 0.92 (3H, t, J 7.5, 7- H_3); δ_{C} (125 MHz, CDCl_3) 171.6 (C=O), 171.2 (C=O), 146.7 (C-4), 137.1 (C-3), 131.5 (C-2), 129.2 (C-1), 31.7 (C-5), 22.2 (C-6), 14.0 (C-7).

(*E*)-3-Butylidene-4-methylenedihydrofuran-2,5-dione **34**

Diacid **33** (75 mg, 0.41 mmol) in dry DCM (2 mL) was cooled to -20 °C, then acetyl chloride (0.1 mL, 6 equiv.) added dropwise. The temperature was maintained at -20 °C for 96 h, after which the solvent was removed under first a stream of N_2 then *in vacuo*, maintaining an internal temperature of -30 °C. Pre-cooled CDCl_3 (2 mL) was added, with an aliquot (0.8 mL) then removed after 0.25 h for NMR analysis. δ_{H} (500 MHz, CDCl_3) 7.24 (1H, t, J 7.5, 4-H), 6.59 (1H, s, 1-HH), 6.13 (1H, s, 1-HH), 2.52 (2H, q, J 7.5, 5- H_2), 1.68 (2H, q, J 7.5, 6- H_2), 1.04 (3H, t, J 7.5, 7- H_3); δ_{C} (125 MHz, CDCl_3) 163.9 (C=O), 163.8 (C=O), 149.3 (C-4), 130.4 (C-3), 125.3 (C-1), 122.3 (C-2), 32.0 (C-5), 21.6 (C-6), 14.0 (C-7). Compound rapidly decomposes to multiple products. IR and MS could not be collected.

(*E*)-2-(Hex-1-en-1-yl)benzo[d][1,3,2]dioxaborole **36**

1-Hexyne (3.5 mL, 2.50 g, 30.5 mmol) was dissolved in THF (5 mL) and *N,N*-dimethylacetamide (0.2 mL) added. Catecholborane (3.9 mL, 3.67 g, 30.5 mmol, 1 equiv.) was added dropwise, and then the reaction heated to reflux for 3 h. The reaction mixture was cooled to room temperature, concentrated *in vacuo*, then purified by column chromatography using ethyl acetate/petrol (10-50% ethyl acetate) to give catecholboronic ester **36** as a yellow oil (4.22 g, 69%). δ_{H} (400 MHz, CDCl_3) 7.24-7.20 (2H, m, Ar-H), 7.10-7.05 (2H, m, Ar-H), 6.86 (1H, m, 2-H), 5.80 (1H, dd, J 18.0, 1.5, 1-H), 2.30 (2H, tdd, J 8.0, 6.0, 1.5, 3- H_2), 1.54-1.45 (2H, m, 4- H_2), 1.44-1.35 (2H, m, 5- H_2), 0.94 (3H, t, J 7.5, 6- H_3); δ_{C} (100 MHz, CDCl_3) 158.2 (C-2), 148.4 (Ar), 122.6 (Ar), 121.4 (C-1), 115.6 (C-1), 112.4 (Ar), 35.9 (C-3), 30.4 (C-4), 22.4 (C-5), 14.1 (C-6). NMR data in accordance with literature.⁴⁰

Diethyl 2-((*E*)-hex-1-en-1-yl)-3-methylmaleate **37**

Potassium carbonate (1.60 g, 11.5 mmol, 1.8 equiv.), triphenylphosphine (110 mg, 0.42 mmol, 0.08 equiv.) and palladium(II) acetate (47 mg, 0.20 mmol, 0.03 equiv.) were dissolved in absolute ethanol (40 mL). Solutions of unpurified vinyl iodide **35** (2.84 g) and boronic ester **36** (1.566 g) each in ethanol (10 mL) were added sequentially, and the resulting dark brown suspension was heated to reflux at 80 °C for 16 h. The dark mixture was then cooled to room temperature, poured into water (30 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with water (2 × 25 mL) and brine (30 mL), dried over MgSO₄, filtered, and concentrated *in vacuo*. The crude material was purified by column chromatography using diethyl ether/petrol (5-20% diethyl ether) to give diester **37** (1.012 g, 40% over two steps). δ_{H} (400 MHz, CDCl₃) 6.31 (1H, dt, *J* 16.0, 1.5 Hz, 4-H), 5.94 (1H, dt, *J* 16.0, 7.0 Hz, 5-H), 4.29 (2H, q, *J* 7.0 Hz, OCH₂), 4.17 (2H, q, *J* 7.0 Hz, OCH₂), 2.18 (2H, q, *J* 7.0, 6-H₂), 1.97 (3H, s, 1-H₃), 1.44-1.35 (2H, m, 7-H₂), 1.34-1.23 (8H, m, 8-H₂, 2 × OCH₂CH₃), 0.88 (3H, t, *J* 7.0, 9-H₃); δ_{C} (100 MHz, CDCl₃) 169.1 (C=O), 167.2 (C=O), 141.5 (C-2), 141.1 (C-4), 124.2 (C-3), 124.0 (C-5), 61.2 (OCH₂), 61.1 (OCH₂), 33.4 (C-6), 30.9 (C-7), 22.3 (C-8), 14.2 (OCH₂CH₃), 14.2 (OCH₂CH₃), 13.9 (C-9), 13.6 (C-1); ν_{max} (film) 2981, 2932, 1721, 1368, 1259, 1181, 1108, 1033; ; HRMS (ESI) calc for C₁₅H₂₄NaO₄ [M+Na]⁺ 291.1567, found 291.1571.

(*E*)-3-Methyl-4-(hex-1-en-1-yl)furan-2,5-dione **38**

Diester **37** (750 mg, 2.8 mmol) was dissolved in ethanol (35 mL) and sodium hydroxide (2 M, 45 mL) added. The resulting solution was stirred at room temperature for 16 h, then sodium sulfate (4%, 50 mL) added and the reaction mixture acidified to pH 1 with hydrochloric acid (6 M). The aqueous mixture was extracted with diethyl ether (4 × 50 mL), and the combined organic layers washed with water (50 mL), then dried over MgSO₄, filtered, and then concentrated *in vacuo* to give maleic anhydride **38** (1.49 g, 91%). δ_{H} (400 MHz, CDCl₃) 7.12 (1H, dt, *J* 16.0, 7.0, 5-H), 6.23 (1H, dt, *J* 16.0, 1.5, 4-H), 2.28 (2H, qd, *J* 7.0, 1.5, 6-H₂), 2.11 (3H, s, 1-H₃), 1.53-1.43 (2H, m, 7-H₂), 1.41-1.31 (2H, m, 8-H₂), 0.92 (3H, t, *J* 7.5, 9-H₃); δ_{C} (100 MHz, CDCl₃) 166.5 (C=O), 164.7 (C=O), 148.2 (C-5), 137.4 (C-3), 135.0 (C-2), 117.3 (C-4), 34.3 (C-6), 30.7 (C-7), 22.4 (C-8), 14.0 (C-9), 9.4 (C-1); HRMS (ESI) calc for C₁₁H₁₄NaO₃ [M+Na]⁺ 217.0835, found 217.0840.

(±)-(5*S*,6*S*,10*aR*,*E*)-5,6-dibutyl-10*a*-methyl-5,6,10*a*,11-tetrahydro-1*H*-cyclonona[1,2-*c*:4,5-*c'*]difuran-1,3,8,10(4*H*)-tetraone **39**

To MgCl₂ (13 mg, 0.14 mmol, 0.5 equiv.) in dry DMSO (3.6 mL) was added anhydride **38** (50 mg, 0.26 mmol) and triethylamine (23 μ L, 17 mg, 0.17 mmol, 0.65 equiv.). The purple solution was stirred at room temperature for 16 h, then quenched by the addition of hydrochloric acid (5 mL, 3 M). The orange solution was extracted with diethyl ether (3 × 20 mL), then the combined organic layers were washed with water (20 mL) and brine (20 mL). After drying over MgSO₄, filtration, and concentration *in vacuo*, the crude material was purified by column chromatography using diethyl ether/petrol/formic acid (10-30% ethyl acetate, 2% formic acid) to give **39** as a yellow oil (5 mg, 10%). δ_{H} (500 MHz, CDCl₃) 6.93 (1H, d, *J* 11.5, 6-H), 3.26 (1H, d, *J* 13.5, 9-*HH*), 2.77 (1H, dd, *J* 13.5, 3.5, 12-*HH*), 2.63 (1H, d, *J* 13.5, 9-*HH*), 2.35 (1H, dd, *J* 13.5, 5.0, 12-*HH*), 1.99-1.86 (2H, m, 5-H and 13-H), 1.75-1.60 (2H, m, 4-*HH* and 14-*HH*), 1.53 (3H, s, 18-H₃), 1.38-1.12 (10H, m, 4-*HH*, 14-*HH*, 2-H₂, 3-H₂, 15-H₂, 16-H₂), 0.97 (3H, t, *J* 7.5, 1-H₃), 0.86 (3H, t, *J* 7.5, 17-H₃); δ_{C} (125 MHz, CDCl₃) 173.8 (C=O), 165.6 (C=O), 165.3 (C=O), 164.2 (C=O), 152.3 (C-6), 147.3 (C-10), 141.4 (C-11), 129.9 (C-7), 48.8 (C-8), 43.1 (C-5), 32.9 (C-9), 31.7 (C-4), 30.3 (C-15), 30.2 (C-3), 29.6 (C-14), 23.2 (C-16), 22.9 (C-2), 20.3 (C-18), 14.2 (C-17), 14.0 (C-1).

(E)-3-Hexylidene-4-methylenedihydrofuran-2,5-dione **40**

Diacid **24** (55 mg, 0.25 mmol) in dry DCM (2 mL) was cooled to $-30\text{ }^{\circ}\text{C}$, then acetyl chloride (0.1 mL, 6 equiv.) added dropwise. The temperature was maintained at $-30\text{ }^{\circ}\text{C}$ for 96 h, after which the solvent was removed under first a stream of N_2 then *in vacuo*, maintaining an internal temperature of $-30\text{ }^{\circ}\text{C}$. Pre-cooled carbon tetrachloride (2 mL) was added, with an aliquot (0.8 mL) then removed after 0.25 h for NMR analysis. The remaining solution was concentrated *in vacuo* at $-30\text{ }^{\circ}\text{C}$ and used immediately without further purification. δ_{H} (500 MHz, CCl_4) 7.10 (1H, br. s, 4-H), 6.50 (1H, br. s, 1-HH), 6.04 (1H, br. s, 1-HH), 2.70-1.90 (2H, m, 5-H₂), 1.75-1.08 (6H, m, 6-H₂, 7-H₂, 8-H₂), 1.00-0.76 (3H, m, 9-H₃); δ_{C} (125 MHz, CCl_4) 162.3 (C=O), 162.2 (C=O), 147.1 (C-4), 130.7 (C-3), 123.4 (C-1), 122.4 (C-2), 31.4 (C-6, C-7 or C-8), 29.6 (C-5), 27.7 (C-6, C-7 or C-8), 22.3 (C-6, C-7 or C-8), 13.8 (C-9); δ_{H} (400 MHz, CDCl_3) 7.21 (1H, t, *J* 7.0, 4-H), 6.56 (1H, s, 1-HH), 6.12 (1H, s, 1-HH), 2.52 (2H, q, *J* 7.0, 5-H₂), 1.66-1.57 (2H, m, CH₂), 1.40-1.20 (4H, m, 2 \times CH₂), 0.93-0.85 (3H, m, 9-H₃) Compound rapidly decomposes to multiple products, including **38**. IR and MS could not be collected.

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